# Comparison of Arterial Spin Labelling and R2* as Predictive Response Biomarkers for Vascular Targeting Agents in Liver Metastases

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**Introduction**: Metastatic liver disease is the main cause of mortality in colorectal carcinoma (CRC) patients, with a 5 year survival rate of 40% following surgical resection of metastases[1]. Surgery with curative intent is only possible in 10-20%[1] of patients, demonstrating the need for alternative therapeutic approaches. The vascular disrupting agent OXi4503 is a compound that targets tumour vasculature and causes central tumour necrosis[2] leaving a small viable rim of tumour cells[3]. The acute (within 4 hours) accumulation of paramagnetic deoxyhaemoglobin resulting from vascular disruption has allowed $R_2$* changes to be used as a biomarker of therapeutic effect [4]. However, Arterial Spin Labelling (ASL) could offer an alternative quantifiable technique for assessing response, by measuring acute changes in tumour perfusion using wholly endogenous contrast mechanisms [5]. The current study therefore aims to compare changes in R2* and ASL following OXi4503 treatment in a preclinical liver metastasis model.

**Method**: *Animal model:* The CRC cell line SW1222 was injected intrasplenically at a concentration of $1x10^6$ cells in 100 µl in serum free media into n=6 MF1 *nu/nu* mice. Cells were allowed to wash through to the liver for 1 minute followed by splenectomy. Solid tumour deposits developed within the liver at ≈4 weeks following surgery.
*MRI:* A 9.4T Agilent VNMRS 20cm horizontal bore system with a 39mm birdcage coil was used, with a warm air blower to maintain animal temperature. Respiratory gating (SA instruments, New York, USA) was used on all scans. Fast spin echo images were used to define a suitable imaging slice within the liver followed by a segmented FAIR Look-Locker ASL sequence with a single slice spoiled gradient readout [5]. R2* values were assessed by a multi-gradient echo (MGE) image sequence covering the entire liver. *FAIR Look-Locker ASL sequence parameters:* 30 x 30mm FOV, 128x128 matrix, TE: 1.18 ms, TI: 110 ms, $TR_{RF}$: 2.3 ms, $TR_I$: 13 s, 50 inversion recovery readouts. Localised inversion thickness: 6 mm, imaging readout slice thickness: 1 mm, 4 lines per segmented acquisition. *MGE sequence parameters:* 8 echoes, $TE_1$=2ms, echo spacing=2ms, TR=280ms; 128x128 matrix, 40x40mm FOV, 1mm slice thickness.
*Dosing:* Cannulation of the tail vein was performed prior to baseline scans. Dosing of 40mg/kg via this remote i.v. line was performed in the scanner bore after baseline scans, and data acquired at 90min post dose.
*Data analysis:* n=18 metastases were evaluable across the n=6 mice for ASL and n=12 were available for R2* analysis. Perfusion maps were generated using the Belle model[7], ($T1_{blood}$ =1.9 s [8], blood-tissue partition coefficient λ=0.95 ml/g [9]) in MATLAB and R2* maps were created using IDL.

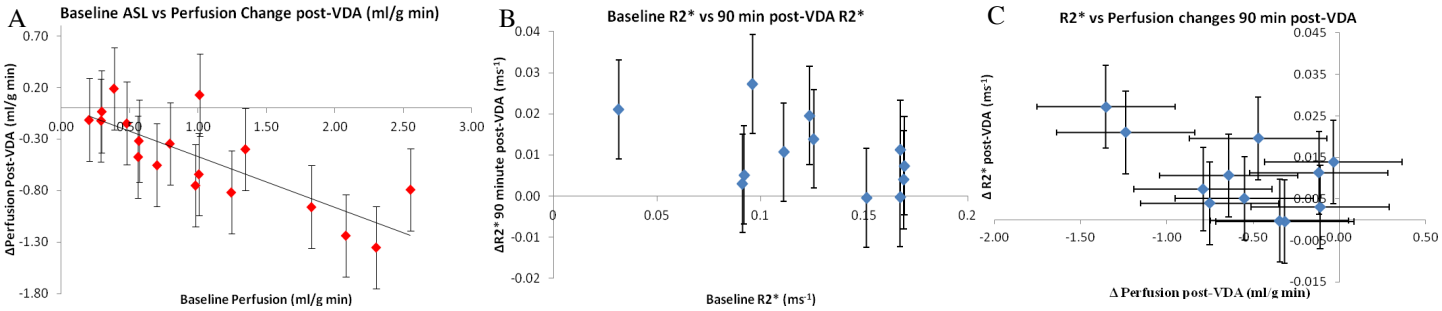


**Fig 1**: Plots showing the liver metastases response 90 minutes post OXi4503 against baseline measurements for perfusion (A) and R2* (B). A significant trend can be seen in the perfusion changes compared to the initial perfusion, however no trend can be seen in the post dose vs baseline R2*. No significant correlation can be seen between the change in R2* and perfusion post dose (C).

**Results**: A significant decrease was measured in ASL measurements of tumour perfusion at 90 mins following OXi4503 administration ($P < 0.01$, Mann-Whitney U test), with a mean change of -0.49 ml/g/min (-43%). A significant correlation was observed between baseline perfusion and the change in perfusion following therapy (Fig. 1A), suggesting that tumours better perfused at baseline responded better to the therapy. A significant increase in R2* was also measured ($P < 0.01$, Mann-Whitney U test), with a mean change of 0.010 $ms^{-1}$ (13%), but with no significant correlation with initial R2* (Fig.1B). There was no significant correlation between ASL and the R2* responses (Fig.1C).

**Discussion**: We were able to detect acute changes in tumour pathophysiology caused by OXi4503 with both ASL and R2*, with a significant decrease in mean perfusion and increase in $R_2$*. This is consistent with the mechanism of action of VDAs: cessation of blood flow leads to a reduction in tumour perfusion and an increase in paramagnetic deoxygenated haemoglobin. Changes in $R_2$* and perfusion were not correlated, indicating a complex relationship between changes in flow and accumulation of deoxyhaemoglobin, which may be specific to individual tumours.
The data presented here shows that ASL can be a predictor of vascular targeting agent efficacy in liver metastases, suggesting that tumour deposits better perfused at baseline display a greater acute response. R2* response was not suggestive of any prognostic ability, but did respond positively. Given the mechanism of action of vascular disrupting agents, ASL provides response biomarkers that afford a less ambiguous interpretation than intrinsic susceptibility ($R_2$*) measures. However, an approach combining the two may provide deeper insights in to the mechanics of tumour response *in vivo*, by relating flow changes to changes in blood oxygen saturation.
The detection of a variable response, even in tumour deposits within the same liver highlights the need for robust assessment of response within individual patients. ASL sequences are non-invasive and do not require the administration of a contrast agent and so could be performed serially, soon after therapy to inform on drug efficacy. Given that brain and kidney FAIR ASL is commonplace in clinical scanners we anticipate a translation of hepatic ASL should be straightforward. Further work will characterise the response at later time points post OXI4503 and assess changes in perfusion and R2* in other tumour lines in preclinical metastases models.

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